Caloplaca epigaea n. sp. from arid soil in Spain and dead grass in Germany

Søchting, Ulrik; Huneck, S.; Etayo, J.

Published in:
Bibliotheca Lichenologica

Publication date:
2007

Document version
Publisher's PDF, also known as Version of record

Lichenologische Nebenstunden – Contr. to lichen taxonomy and ecology in honour of Klaus Kalb.
A. Frisch, U. Lange & B. Staiger (eds): Bibliotheca Lichenologica 96: 279–286.
J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin und Stuttgart, 2007.

Caloplaca epigaea sp. nova from arid soil in Spain and dead grass in Germany

Ulrik Søchting[1], Siegfried Huneck[2] & Javier Etayo[3]

[1] Section of Microbiology, Department of Biology, University of Copenhagen, O. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark (ulriks@bi.ku.dk)

[2] Fliederweg 34a, D-06179 Langenbogen/SK, Germany

[3] Navarro Villoslada 16, 3° dcha, 31003 Pamplona, Navarra, Spain

Abstract: A *Caloplaca* species from summer warm, arid soil and dead plant material in Spain and Germany is characterized by the presence of a well developed thallus and ascospores with thin septum. Molecular data show it to be related to the *C. lactea* group where such a combination of characters was unknown. It is described here as *C. epigaea*.

Key words: *Caloplaca epigaea*; *C. lactea* group; Spain; Germany; arid soil; gypsum; molecular phylogeny

Introduction

The advent of molecular information has provided the ability to relate taxa that would otherwise not be easily combined due to very distant provenience or growth on different substrates. Two morphologically similar collections of *Caloplaca* originating from gypsum soil in Spain and from detritus on old mine tips in Central Germany, respectively, proved by blast search to have very similar ITS and identical mitochondrial 18S ribosomal sequences, which indicates that they belong to a single taxon. A subsequent alignment and phylogenetic analysis with molecularly related taxa showed the species to belong in the *C. lactea* group. This relationship is supported by the narrow septum of the ascospores, a characteristic of this group. However, as it does not conform to any known species of this group it is described below as new, with the name *C. epigaea*.

Methods

Sections for microscopy were made with a Reichert-Jung Cryostat 2800 Frigocut-E. and studied by standard microscopy in water with an Olympus BX60 light microscope. Photos were taken with a Nikon DS-5Mc digital camera. Secondary chemistry was analyzed with HPLC according to Søchting (1997).

DNA was extracted using the DNeasy Plant Mini Kit ™ (Qiagen). PCR amplification of part of the mitochondrial 18S ribosomal DNA region was performed according to Schmitt et al. (2003) using the primers mrSSU1 (Zoller et al. 1999) and MSU7 (Zhou & Stanosz 2001). The PCR products were subsequently

Tab. 1 Voucher source information and GenBank accession numbers for mitochondrial ribosomal 18S DNA sequences.

Taxon	Locality, date, collector and herbarium	GenBank no.
C. crenulatella	Austria. Steiermark, Eisenerz, 17. viii. 1998, Søchting 9359 [C]	EU088308
C. epigaea	Spain. Aragón, Zaragoza, Monegros, Bujaraloz, Javier Étayo 21453 [JACA]	EU088314
C. epigaea	Germany. Sachsen-Anhalt, Mansfelder Land bei Hettstedt, 11. x. 2006, Huneck [hb Huneck]	EU088315
C. marina	Iceland. S-Múlassýsla, Bøjarstadir, Søchting 7520 [C]	EU088309
C. microthallina	Sweden. Halland, Hallands Väderö, Søchting 7480 [C]	EU088310
C. ochracea	France. Gard, Sauveterre, Truel, 6. vii. 1998, C. Roux [C]	EU088311
C. regalis	Chile. Region de Magellanes, Pali-Aike, Elvebakk 98:349 [TROM]	EU088312
C. scopularis	Iceland. S-Múlassýsla, Bøjarstadir, Søchting no. 7521 [C]	EU088313
C. thuringiaca	Germany. Thuringia, Kyffhäuser Mts., 18. vi. 2005, [Topotype, Lich. Sel. Exsic. R. Stordeur no. 2]	EU088316

cleaned using Nucleofast® (Macherey-Nagel). Sequencing was performed by Macrogen Ltd., Korea, using the same primers as used for amplification. Sequence chromatograms were edited and assembled using Sequencher software version 3.1.1 (GeneCodes, Ann Arbor, Michigan). The sequences were aligned with Sequencher and subsequently adjusted manually by visual inspection. The taxon sampling consisted of eight *Caloplaca* taxa, including two specimens of *C. epigaea* from both habitats. *C. scopularis* was used as outgroup (tab. 1). *C. thuringiaca* and *C. regalis* were included because they represent groups close to the *C. lactea* group.

Phylogenetic analysis of the mtSSU dataset was conducted using PAUP* 4.0b10 on a Macintosh platform (Swofford 2003). 728 characters were constant, 20 characters were parsimony uninformative and 30 characters were informative. The heuristic search for the best tree was performed with 1000 random addition sequences (RAS), tree-bisection-reconnection (TBR) swapping, MultTrees in effect, branches

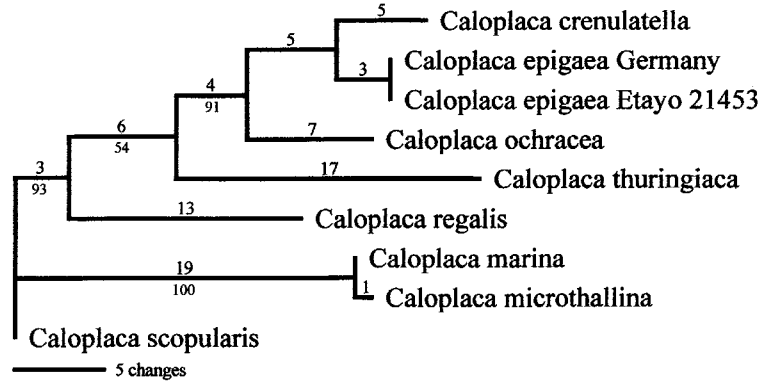

Fig. 1 Phylogenetic tree based on mitochondrial 18S rDNA. One of four most parsimonious trees presenting the position of *Caloplaca epigaea* and the *C. lactea* clade (*C. crenulatella*); numbers below branches are bootstrap values.

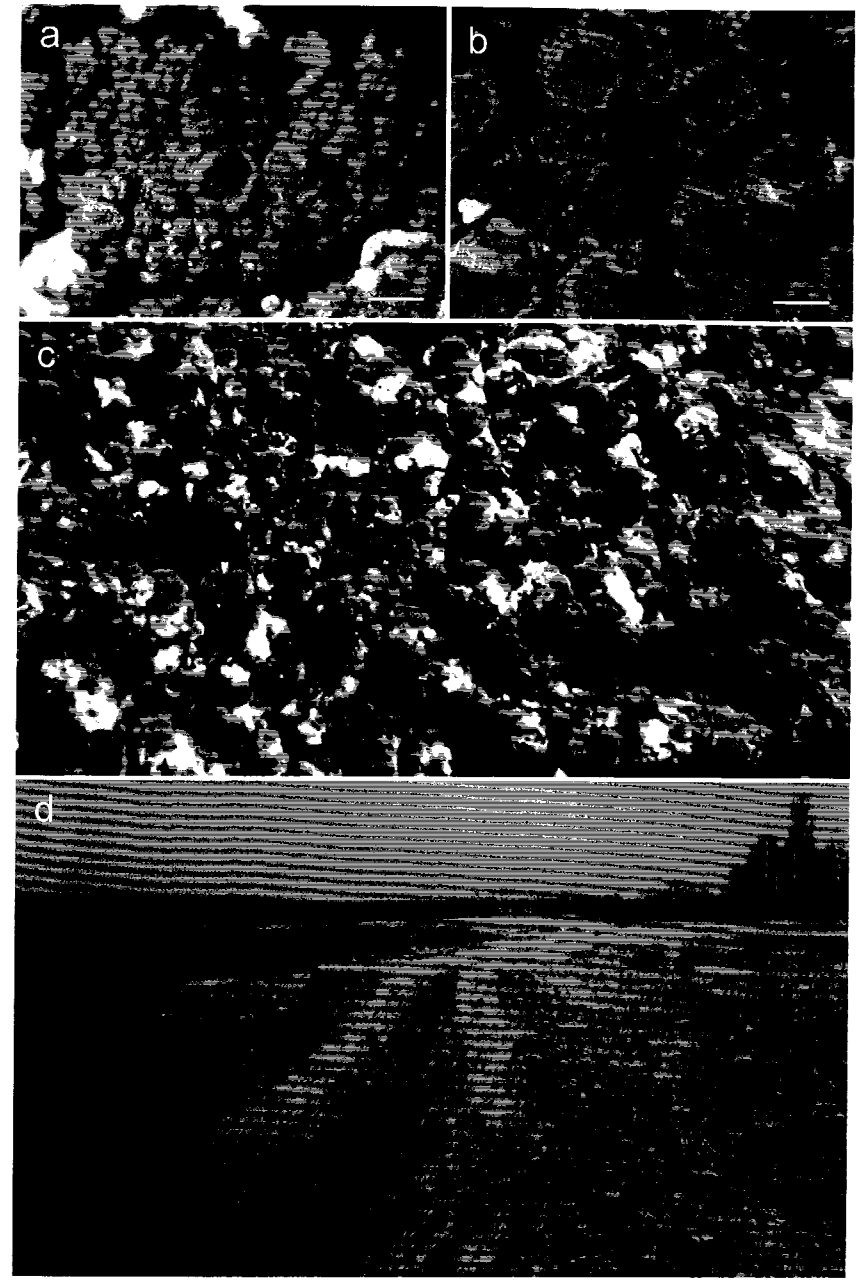

Fig. 2 *Caloplaca epigaea*. (a, b) Etayo no. 21453 (holotype); (c) specimen from Hettstedt (Huneck, 11. x. 2006); (d) German locality of *C. epigaea*: Hettstedt, copper slate tip with *Festuca* tussocks; scale bars: a, b = 0.5 mm.

collapsed if maximum branch length is zero; gaps were treated as 'fifth base'. The optimization criterion used was maximum parsimony, with 1000 bootstrap replicates completed, and 1 RAS per bootstrap replicate. Tree length was 63. One of four most parsimonious trees is shown in fig. 1.

Results

Caloplaca epigaea Søchting, Huneck & Etayo, sp. nova

Caloplacae interfulgens similis, sed apothecia majores et thallus ad terrum crescens.

Type: Spain. Aragón, Zaragoza, Monegros, Bujaraloz, cerca del hostal El Ciervo, lomas yesosas en el margen de la carretera, 400 m, sobre 'suelo yesoso', 30T 0749034, 459402520, 20. iii. 2004, A & J. Etayo no. 21453 [JACA–holotype; C, hb Etayo–isotypes].

(Figs 2, 3)

Thallus crustose, effuse, soon covered by irregular, strongly convex to semiglobular, crowded areoles up to 0.4 mm broad. *Margin* slightly effigurate to disappearing (fig. 2a). *Colour* orange to yellowish orange. *Apothecia* numerous, sessile, gradually covering most of the thallus (figs 2b, c), lecanorine to zeorine, up to 0.5–1 (–1.3) mm diam. *Disc* plane, orange, with fine epipsamma. *Margin* prominent, persistant, *c.*150 µm thick, even or carved, lighter orange than the disc.

Thalline exciple with few to numerous algae. *Proper exciple* consisting of a fan-shaped prosoplectenchymatic tissue of narrow, up to 5 µm long cells. *Cortex* of excipulum consisting of perpendicular hyphae terminating at the surface in slightly elongated, 9–11 µm long cells that are 5–6 µm in diam. and slightly protruding to form a surface composed of semiglobular individual cell surfaces which are covered by anthraquinone crystals (fig. 3a). *Hypothecium* with lipid droplets. *Hymenium* 80–100 µm high. *Paraphyses* 1.5 µm thick, apically sometimes branched, with swollen, up to 5 µm thick apical cells. *Asci* with up to 8 spores, but frequently some of them are deformed or missing. *Spores* hyaline, polarilocular, 16.5–20 × 6.5–9 µm; septum 1–1.5 µm (figs 3b, c).

Pycnidia: Not seen.

Chemistry: Dominance of parietin with a smaller proportion of emodin, teloschistin, fallacinal and parietinic acid (Chemosyndrome A of Søchting (1997)). All yellow or orange parts react K + red.

Ecology: *C. epigaea* in Spain is a rare lichen on gypsic soil, an exposed soil typical of the Ebro Depression, where it has been found in three provinces: Huesca, Navarra and Zaragoza. It grows under arid conditions with other lichens of the multicoloured lichen community ('*Die bunte Erdflechtengesellschaft*') typical of xerophilous grass *Gypsophiletalia* vegetation, like *Acarospora nodulosa*, *A. placodiiformis*, *Diploschistes diacapsis*, *Endocarpon loscosii*, *Fulgensia desertorum*, *F. poeltii*, *Psora decipiens*, *P. saviczii*, *Squamarina cartilaginea*, *S. lentigera* and *Toninia sedifolia*. Other species more rarely associated with it are: *Buellia epigaea*, *B. zoharyi* and *Teloschistes lacunosus*. The recently described *Lecidea circinarioides* grows on mineral gypsum near these communities.

Although the third author has explored many localities with the above characteristics in the Ebro Depression, the species was only seldom found and seems to be

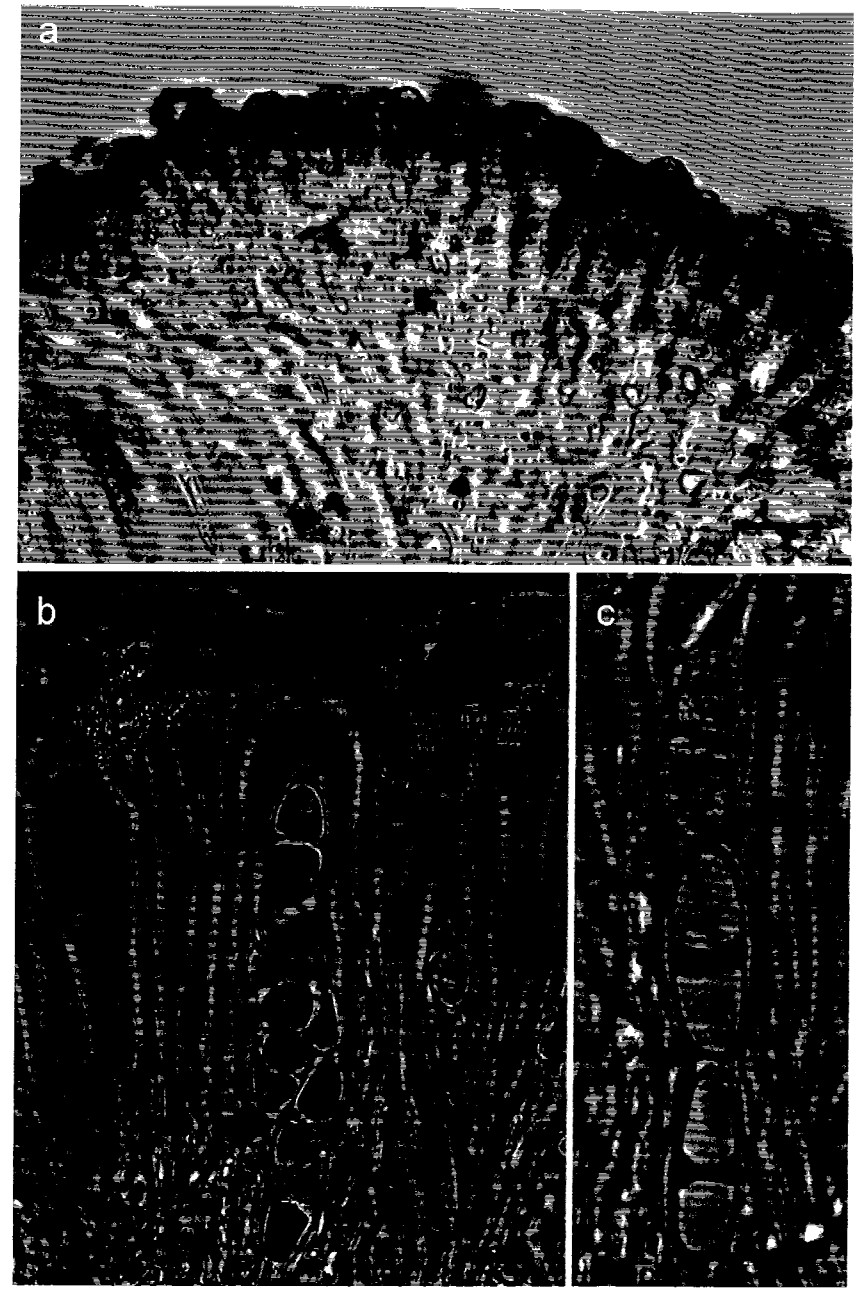

Fig. 3 *Caloplaca epigaea* (Etayo no. 21453, holotype). (a) Cortex of proper excipulum; (b) hymenium with asci and spores; (c) ascospores; scale bars = 10 µm.

very rare, occurring only in very rich localities with a high coverage of those lichens and with abundant lichenicolous fungi. The type locality is the only one in which it has a large dispersed population mixed with the lichens listed above. Another species that seems to need similar requirements and is also rare in this habitat is *Buellia zoharyi*.

In Central Germany *Caloplaca epigaea* was collected from the base of dead grass among Permian limestone (Zechsteinkalk) (fig. 2). The German habitat belongs to the Mansfeld basin, which is situated at the rain shadow of the Harz Mountains with precipitation amounting to only 480–500 mm (Huneck 2006). *C. epigaea* is very rare (about 10 thalli) and has been found hitherto only at the plateau of the copper slate tip Eduard where it grows at the more or less dust impregnated lower parts of dead *Festuca*, up to about 5 cm above the ground. The thalli were only found on the southern part of the tussocks. The ground consists of calcareous soil (Zechstein).

Based on the few known localities it appears that *C. epigaea* is a species of alkaline soils and more or less soil impregnated detritus in summer warm sites with low precipitation. It is very likely that the recognition of the species will bring to light more collections, particularly from the understudied arid regions of the Near East that will improve the understanding of its ecology.

Additional specimen studied: **Germany.** Sachsen Anhalt, Mansfeld county, on dead grass (*Festuca*) in the southern part of the copper slate tip (plateau) of the former mine Eduard near Hettstedt, on Zechstein in sunny position, 11. x. 2006, Huneck [hb Huneck] (fig. 2). **Spain.** Navarra, Ablitas, Peñadil y El Montecillo, lomas yesosas con algún pino, sobre suelo yesoso, 41° 57′ 21,5″ N 01° 37′ 54,7″ W, 15. v. 2005, J. Etayo no. 22381 [hb Etayo]. Aragón, Huesca: Monegros, Cercanías de Serreta Negra, entre Caspe y Candasnos, en suelo yesoso, 350 m, 20. iii. 2004, J. & A. Etayo no. 21516 [hb Etayo].

Discussion

The two specimens of *C. epigaea* from Germany and Spain, respectively, have completely identical base sequences in the mitochondrial ribosomal 18S gene. This indicates a close relationship, but not necessarily conspecificity, as some closely related species in the family Teloschistaceae can have identical base sequences in the mitochondrial ribosomal 18S gene (unpublished results). Nuclear ribosomal ITS sequences (not presented) are 98% identical, and are thus supporting the close relationship between the two populations. Infraspecific variation in the ITS region is not unusual as has been demonstrated, e.g. for *Xanthoria parietina* (Murtagh et al. 2002, Lindblom & Ekman 2005).

In spite of the difference in substrate we consider the two collections to represent one species as there are no significant morphological or anatomical differences between them, and the molecular data are not contradictive to this conclusion. However, it is possible that further studies including more collections may show the two different haplotypes with possibly slightly differing ecology to represent separate subspecies or species.

The molecular data as illustrated in the phylogenetic tree (fig. 1) show that *C. epigaea* belongs in the neighborhood of the *C. lactea* group of the subgenus *Gyalolechia* (A. Massal.) Boistel represented by *C. crenulatella*. This group was critically studied by Navarro-Rosinés & Hladun (1996), who included seven taxa,

viz.: *C. aquensis*, *C. crenulatella*, *C. ferrari*, *C. interfulgens*, *C. lactea*, *C. lacteoides* and *C. marmorata*. The *C. lactea* group, which has not been shown to be monophyletic, consists of non-lobate *Caloplaca* species on calciferous rocks of Mediterranean and central European distribution having spores with narrow septa that are normally less than 3 µm thick. All species have the same chemosyndrome A (Søchting, unpublished), which is the most common syndrome within the genus. The narrow septum in the ascospore is characteristic for members of the subgenus *Gyalolechia* (Clauzade & Roux 1985), which is most certainly polyphyletic. In addition to the *C. lactea* group the subgenus includes the bryophilic and organophilic species *C. nivalis* and *C. livida*, which, however, grow on acid substrates in arctic-alpine regions. *C. epigaea* conforms to all the characteristics of the *C. lactea* group except that it grows on soil and detritus. A well-developed and epilithic thallus is absent from all the species in the *C. lactea* group as circumscribed by Navarro-Rosinés & Hladun (1996), except for *C. interfulgens* (Nyl.) Steiner. This is a rarely collected species described from North Africa, which, according to Navarro-Rosinés & Hladun (1996) has not been convincingly reported from Europe.

There are other species of *Caloplaca*, which have been published growing on soil in arid climates. Some of them may actually also be related to the *C. lactea* group. They are: *C. geoica* H. Magn., *C. geogena* (Vain.) Zahlbr., *Placodium geophilum* Räsänen, *C. psammophila* Poelt & Hinter., *C. terrigena* (Räsänen) H. Magn. and *C. tominii* Savicz.

C. geoica described by Magnusson (1940) from Central Asia is lobate at the circumference and has smaller spores (12–14 × 5.5–7 µm) with 1–1.5 µm thick septum. The lobate thallus and the shorter spores separate it from *C. epigaea*.

C. geogena is a sterile species only known from Chokotka, NE-Russia.

Placodium geophilum described from Russia has according to Magnusson (1940) 'a hardly perceptible, grayish white film upon the earth round the apothecia'. Its spores are 12–13.5 × 6.5–7 µm with a 2–3 µm thick septum, and the terminal cells of the paraphyses are unusually broad, 6.5–9 µm. The lacking thallus and the shorter spores separate it from *C. epigaea*.

C. psammophila was described from the Karakorum in the Himalayas at 2750 m from dry rocky slopes in xeric habitats (Poelt & Hinteregger 1993). It is only known from the type collection, which we have compared with *C. epigaea*. The spores are similar to those of *C. epigaea*, but the thallus is more submerged in the substrate. Furthermore, the very regular apothecia separate it from *C. epigaea*, where the apothecial margin is thick and irregular.

Placodium terrigenum was described by Räsänen (1939) from California, and it was combined into *Caloplaca* by Magnusson (1940). It differs from *C. epigaea* in e.g. having a 'hardly perceptible, sordid ochraceous yellow, contiguous, thin, smooth, non-areolate thallus' (Magnusson 1940). The spores are 15–17 × 6–7 µm with a thin (1.5–2 µm) septum.

C. tominii is characterized by a scutate thallus with soralia on the lower side and at the margins. It is very rarely fertile.

As pointed out by Poelt & Hinteregger (1993) arid terricolous habitats in central Asia are likely to host many species of *Caloplaca*, but the difficulty of collecting and curating lichens from the biological soil crust in arid regions has

been a complication for their proper description and understanding. Molecular analyses of recent collections from such arid regions may, however, disclose more species, and according to the spore characters of several of the above mentioned species, they could well also belong in the vicinity or in the *C. lactea* group.

Acknowledgements

Lene Christiansen performed the HPLC analyses and Anette Løth assisted with the molecular analyses. They are both warmly thanked. Herbarium BBC is thanked for access to material and herbarium GZU for loan of specimens.

References

CLAUZADE, G. & ROUX, C. (1985): Likenoj de Okcidenta Europo. Ilustrita Determinlibro. – Bull. Soc. Bot. Centre-Ouest, nouv. sér., num. spéc. 7: 1–893.

HUNECK, S. (2006): Die Flechten der Kupferschieferhalden um Eisleben, Mansfeld und Sangerhausen – Mitt. Florist. Kartierung Sachsen-Anhalt, Sonderheft 4: 1–62.

LINDBLOM, L. & EKMAN, S. (2005): Molecular evidence supports the distinction between *Xanthoria parietina* and *X. aureola* (Teloschistaceae, lichenized Ascomycota). – Mycol. Res. 109(2): 187–199.

MAGNUSSON, A. H. (1940): Lichens from Central Asia. – In HEDIN, S. (ed.): Reports Scientific Exped. North-west. provinces of China (the Sino-Swedish expedition), 13, XI. Botany, 1. – Aktiebolaget Thule, Stockholm: 1–168.

MURTAGH, G. J., DYER, P. S., FURNEAUX, P. A. & CRITTENDEN, P. D. (2002): Molecular and physiological diversity in the bipolar lichen-forming fungus *Xanthoria elegans*. – Mycol. Res. 106(11): 1277–1286.

NAVARRO-ROSINÉS, P. & HLADUN, N. L. (1996): Les especies saxícolo-calcícolas del grupo de *Caloplaca lactea* (Teloschistaceae, líquenes), en las regiones mediterránea y medioeuropea. – Bull. Soc. Linn. Provence 47 (1996): 139–166.

POELT, J. & HINTEREGGER, E. (1993): Beiträge zur Kenntnis der Flechtenflora des Himalaya. VII Die Gattungen *Caloplaca*, *Fulgensia* und *Ioplaca*. – Biblioth. Lichenol. 50: 3–247.

RÄSÄNEN, V. (1939): II. Contribucion a la flora liquenologica sudamericana (Beiträge zur Flechtenflora Südamerikas II). – Anales Soc. Ci. Argent. 128: 133–147.

SCHMITT, I., LUMBSCH, H. T. & SØCHTING, U. 2003. Phylogeny of the lichen genus *Placopsis* and its allies based on Bayesian analyses of nuclear and mitochondrial sequences. – Mycologia 95: 827–835.

SWOFFORD, D. L. (2003): PAUP*, Phylogenetic analysis using parsimony (*and other methods). Sunderland, Massachusetts: Sinauer Associates.

SØCHTING, U. (1997): Two major anthraquinone chemosyndromes in Teloschistaceae. – Biblioth. Lichenol. 68: 135–144.

ZHOU, S. & STANOSZ, G. R. (2001): Primers for amplification of mtSSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. – Mycol. Res. 105: 1033–1044.

ZOLLER, S., SCHEIDEGGER, C. & SPERISEN, C. (1999): PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. – Lichenologist 31(5): 511–516.

Lichenologische Nebenstunden – Contr. to lichen taxonomy and ecology in honour of Klaus Kalb. A. Frisch, U. Lange & B. Staiger (eds): Bibliotheca Lichenologica 96: 287–297. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin und Stuttgart, 2007.

Santessoniella saximontana, a new lichen species from British Columbia

TOBY SPRIBILLE[1], PER MAGNUS JØRGENSEN[2], MATTHIAS SCHULTZ[3] & ISABELLE HOUDE[4]

[1] Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany (tspribi@uni-goettingen.de)

[2] Department of Natural History, Botanical Museum, University of Bergen, Allégaten 41, N-5007 Bergen, Norway

[3] Botanischer Garten und Biozentrum Klein Flottbek, Universität Hamburg, Ohnhorststrasse 18, D-22609 Hamburg, Germany

[4] Department of Forest Sciences, University of British Columbia, 3041–2424 Main Mall, Vancouver, BC, V6T 1Z4, Canada

Abstract: *Santessoniella saximontana* T. Sprib., P. M. Jørg. & M. Schultz, sp. nova is described from bark of *Thuja plicata* from the Rocky Mountains of south-eastern British Columbia. It is similar to *S. luctuosa* but differs in its blackish brown thallus, smaller, convex apothecia and differently shaped, non-ornamented ascospores. A revised key is provided to the species of *Santessoniella* sensu lato in North America.

Key words: Conservation; cyanolichens; key; old-growth forest; Pannariaceae; plasmodesmatoid connections

Introduction

Although botanists have been studying the lichens of British Columbia for over 100 years, the diversity of its lichen flora is only beginning to be appreciated. Several previously unknown species have come to light in recent years from studies of old-growth inland *Thuja plicata-Tsuga heterophylla* rainforests (e.g. *Lobaria silvae-veteris*, GOFFINET & GOWARD 1998; *Spilonemella americana*, HENSSEN & TØNSBERG 2000; *Sticta oroborealis*, TØNSBERG & GOWARD 2001). Since 2004, the first and last authors have been involved in an assessment of lichen diversity in an old-growth *Thuja plicata* forest located in the Albert River watershed along the border of British Columbia and Alberta, south of Banff National Park. This forest is unusual within the inland rainforest formation for several reasons, notably (1) its occurrence at the extreme eastern periphery of this forest type; (2) its isolation of over 100 km and several mountain ranges from the nearest comparable stands, and (3) its occurrence over Devonian limestone (most rainforests in north-western North America occur over acidic parent material). An account of some of the lichen species found during this assessment is provided by HOUDE et al. (2007).